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Yolk androgens do not appear to mediate sexual conflict over parental investment in the collared flycatcher *Ficedula albicollis*

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Abstract

Males and females are in conflict over parental care, as it would be favourable for one parent to shift labour to the other. Yolk hormones may offer a mechanism through which female birds can influence offspring traits in ways that increase the relative investment by the male. We studied the role of yolk androgens in mediating sexual conflict over parental care in the collared flycatcher (*Ficedula albicollis)*. In a cross-fostering experiment, the male's proportion of total feeding visits increased with increasing androgen levels in the foster eggs. This suggests that sexual conflict over parental care may be influenced by the female's differential allocation of yolk androgens or a maternal effect associated with yolk androgens. However, when we experimentally elevated yolk androgen levels, male feeding rates did not differ between control and androgen-manipulated nests. This suggests that other egg components correlated with yolk androgen levels, rather than yolk androgen levels *per se,* may influence male parental effort. In conclusion, yolk androgens *per se* do not appear to mediate sexual conflict over parental investment in the collared flycatcher.

Keywords: hormones, maternal effects, parental care, parental investment, reproductive effort

Introduction

In species with biparental care, females and males are in conflict over the amount of parental care they provide to their offspring (Trivers, 1972; reviewed by Arnquist and Rowe, 2005; Houston et al., 2005; Lessells, 2006; Hartley and Royle, 2007). For each parent, increased investment reduces it's future reproductive success and survival prospects (e.g. Williams, 1966; Roff, 1992; Parker et al., 2002), and therefore it would be favourable for one parent to shift labour to the other (Parker et al., 2002; Houston et al., 2005). The parents' decision about how much to invest in the offspring depends on offspring need and/or brood value, as well as the feeding behaviour of the other parent (e.g. Hinde and Kilner, 2007). A parent could thus try to manipulate the investment of its partner by changing either offspring traits or his or her own behaviour (e.g. Slagsvold et al., 1995; Westneat and Sargent, 1996; Arnquist and Rowe, 2005; Lessells, 2006).

Offspring phenotype is affected by maternal effects (Mousseau and Fox, 1998), for instance via egg composition (e.g. Groothuis et al., 2005a). In some bird species, androgens deposited in the egg yolk have been found to increase competitive ability of chicks (correlative data in Schwabl, 1993; experimental data in Eising et al., 2001), chick begging (experimental data: Schwabl, 1996; Schwabl and Lipar, 2002; Eising and Groothuis, 2003; Boncoraglio et al., 2006; von Engelhardt et al., 2006) and growth (experimental data: Schwabl, 1996; Eising et al., 2001; Pilz et al., 2004; Navara et al., 2005, 2006; Tschirren et al., 2005). In other studies, however, no positive effect of elevated yolk androgens on begging or growth was found (experimental data: Sockman and Schwabl, 2000; Andersson et al., 2004; Pilz et al., 2004; Uller et al., 2005; Saino et al., 2006), or the effect depended on offspring sex (e.g. Saino et al., 2006; Müller et al., 2008; Sockman et al., 2008). The effects of yolk androgens thus seem to vary, but there is evidence that they can affect offspring traits. It has therefore been proposed that by allocating more yolk androgens into the egg, a female could affect offspring trait(s) to which males respond, and this way increase the relative

61 feeding effort of the male to her benefit (Groothuis et al., 2005a; Michl et al., 2005; Gil et al., 2006;
62 Lessells, 2006; Moreno-Rueda, 2007; Müller et al., 2007a).

63 This hypothesis relies on several assumptions. First, yolk androgen levels should
64 affect chick traits to which the parents respond by changing their food provisioning. There is
65 evidence from several species for this assumption (reviewed e.g. by Müller et al., 2007a, see
66 above). However, a direct link between yolk androgen levels and parental feeding effort has been
67 investigated in only one study thus far (Tschirren and Richner, 2008). Second, increasing yolk
68 androgen levels should either impose a cost or be constrained, since otherwise all females could
69 afford to allocate high levels of androgens (e.g. Groothuis et al., 2005a; Groothuis and Schwabl,
70 2008). Indeed, elevated levels of androgens in the yolk can be costly for the chicks, as an
71 immunosuppressant immunosuppressive? effect of androgens has been reported in some studies
72 (e.g. Groothuis et al., 2005b; Müller et al., 2005; Navara et al., 2005; but see Andersson et al 2004;
73 Tschirren et al., 2005; Müller et al., 2007b). Moreover, such elevated levels may require elevated
74 circulating androgen levels in the female, which may, for example, delay or inhibit egg laying (e.g.
75 Clotfelter et al., 2004; Rutkowska et al., 2005; discussed in Groothuis and Schwabl, 2008). Finally,
76 females should be able to respond differently to the chick trait influenced by yolk androgens than
77 males. This assumption is yet to be explored. Two mechanisms are possible, depending on whether
78 both parents or only the male respond to the trait through which the female attempts to manipulate
79 the male (Müller et al., 2007a). 1) If both parents respond to the androgen-mediated offspring trait,
80 males are expected to increase their investment in response to increasing yolk androgen levels
81 deposited in the eggs by the female; females, however, should have lower responsiveness to
82 androgen-mediated chick traits when allocating high androgen levels into their eggs than when
83 allocating low levels. 2) If only males respond to the chick trait (hereafter a *male-specific* trait),
84 males should increase their feeding rate with increasing yolk androgen levels while females should
85 not change their investment. Previous studies have shown that it is possible that females and males

respond to different components of nestling behaviour and phenotype, for example colour (Jourdie et al. 2004, de Ayala et al. 2007, Ewen et al. 2008, Galvan et al. 2008, Tanner and Richner, 2008), size (reviewed in Slagsvold et al., 1997; Lessells, 2002), condition (e.g. Christe et al. 1996) or visual vs. vocal cues of begging (Kilner et al., 2002, reviewed in Müller et al. 2007).

We studied whether yolk androgens could mediate sexual conflict over parental care in a small passerine bird, the collared flycatcher (*Ficedula albicollis*) by conducting two experiments. Firstly, we examined whether naturally varying levels of yolk androgens affect the relative work load of the parents. We did this by cross-fostering full broods with naturally varying yolk androgen levels among nests and monitoring the subsequent parental feeding rates. We predicted that if females can manipulate male investment through differential yolk androgen deposition, and the androgen-mediated nestling trait is male-specific, the proportion of male feeding visits should be positively correlated with the yolk androgen levels of the foster eggs (hereafter foster yolk androgens), but not of original eggs (hereafter original yolk androgens). In contrast, if the manipulation is mediated through an androgen-mediated trait to which both parents respond, we predicted a positive correlation between proportion of male feeding visits and original androgen level, but no correlation with proportion of male feeding visits and foster androgen level. This is because females allocating high yolk androgen levels should have lower responsiveness to androgen-mediated chick traits than females allocating low yolk androgen levels. Secondly, we experimentally increased yolk androgen levels of whole clutches and monitored the subsequent parental feeding rates to test whether yolk androgens *per se* allow females to manipulate males. We predicted that if male manipulation by the female is mediated by yolk androgens *per se*, male feeding rate should be higher in broods of the androgen-manipulated than the control group.

Methods

111 *Study site and study species*

112 The experiments were conducted in spring 2007 on the island of Gotland, Sweden (57°10' N,
113 18°20' E) in a nest box breeding population of collared flycatchers monitored since 1980
114 (Gustafsson, 1989). The study area consists of several small and spatially discrete forest patches.
115 The collared flycatcher is a small (ca. 13 g), migratory, passerine, which breeds in central and
116 eastern Europe and on the islands of Gotland and Öland in Sweden. Average clutch size in this
117 population is six eggs, and average brood size four nestlings.

118

119 *Cross-fostering experiment*

120 Nest boxes were checked every other day starting at end of April to monitor the progress of nest
121 building and egg laying. When one or two eggs were found in the nest, they were marked with non-
122 toxic permanent marker and the nest box was visited the in following day(s) to collect the third egg
123 of the clutch on the day of laying (N = 31 nests). These eggs were taken to the laboratory, and yolks
124 were separated from albumen on the same day and stored frozen at -20˚C until hormone analysis
125 (see below). The collected egg was replaced by a dummy egg in each nest. The average clutch size
126 in this species and population is six eggs, so the third egg is one of the middle eggs of a clutch. In
127 most species studied so far, including the collared flycatcher and its sister species the pied
128 flycatcher (*Ficedula hypoleuca*), the between-clutch variation in yolk androgen levels is higher than
129 the within-clutch variation (e.g. Reed and Vleck, 2001; Groothuis and Schwabl, 2002; Pilz et al.,
130 2003; Tschirren et al., 2004; Michl et al., 2005; Müller et al., 2007b, Tobler et al., 2007; T.
131 Laaksonen, unpubl. data). No within-clutch pattern of yolk androgens was found in a Hungarian
132 population of the collared flycatchers (Michl et al., 2005), thus yolk androgen concentration in the
133 middle egg should be a good proxy for the mean hormone level of a clutch. Even if within-clutch
134 variation in hormone deposition would have occurred in some individuals of our study population,
135 the third egg would still represent the medium concentration of the whole clutch, as long as the

136 pattern of within-clutch variation is linear (as found in many studied species with significant within-
137 clutch variation, e.g. Reed and Vleck, 2001; Groothuis and Schwabl, 2002; Pilz et al., 2003;
138 Tschirren et al., 2004).

139 Nests were monitored to record final clutch size and hatching date as part of the
140 population monitoring. Two days after hatching, complete broods were cross-fostered between
141 sampled nests (matched for hatching date and brood size), so that in most cases the yolk androgen
142 levels of the foster brood (i.e. of the third egg of the foster brood) was known. Both parents were
143 caught in the nest box using a swing-door-trap when feeding 6 to 10 days-old nestlings. They were
144 weighed and measured and their age was determined as yearling or older (see e.g. Pärt and
145 Gustafsson, 1989).

146 Parental feeding frequency was measured at day 6 after hatching by observing
147 parental feeding behaviour from a hide ca. 15 meters distance from the nest. After two feeding visits
148 by the parents (to ensure that they were feeding normally), one hour of feeding visits by the parents
149 was observed. Peak of feeding activity in this species occurs between day 5 and day 10 of nestling
150 rearing. Feeding rate was measured as the number of feeding visits by each parent per hour. Feeding
151 rate has been shown to correlate positively with energy expenditure in this population (Pärt et al.,
152 1992), thus it can be used as a measure of parental investment. Parental feeding rate further
153 correlates with both chick weight and condition at fledging and fledging success (Doligez et al.,
154 2004), and these variables are related to juvenile survival in this species (Linden et al., 1992).
155 Feeding rates were recorded mostly between 8.00- 12.00 am, and only in good weather conditions.

156

157

158 *Yolk androgen analysis*

159 Yolk testosterone (T) and androstenedione (A4) concentrations were analysed by
160 radioimmunoassay (RIA). Yolks were thawed and homogenised with 400 µl of distilled water.

Aliquots of this yolk / water emulsion (approximately 100mg) were mixed with 150 µl of distilled water and 50 µl of 3H Tracer T (ca. 2000 counts per minute) to assess extraction efficiency. The samples were extracted twice with 2.5ml of 70% diethylether / 30% petroleumether (vol : vol) and dried under a stream of nitrogen. The extracts were then re-dissolved in 1ml 70% methanol, centrifuged and decanted. The supernatant was dried under a stream of nitrogen and re-dissolved in PBS. T and A4 were measured in duplicates using DSL (Diagnostic System Laboratories, USA) radioimmunoassay kits following the manufacturer's protocol. The average recovery rate was 72%. The yolks were analysed in two assays with an inter-assay coefficient of variation of 5.9% for A4 and 1.6% for T and an intra-assay coefficient of variation of 10.0% for A4 and 5.7% for T. Parallelism was confirmed, and concentrations of T and A4 were not affected by extraction efficiency (T concentration: $F_{1,21} = 0.01$, $p = 0.94$; A4 concentration: $F_{1,21} = 0.0$, $p = 0.99$; range of extraction efficiency: 66.7 - 76.6 %), egg weight (T: $F_{1,21} = 0.02$, $p = 0.89$; A4: $F_{1,21} = 0.01$, $p = 0.92$), yolk weight (T: $F_{1,21} = 0.69$, $p = 0.42$; A4: $F_{1,21} = 0.06$, $p = 0.80$) or assay (T: $F_{1,21} = 1.70$, $p = 0.21$; A4: $F_{1,21} = 3.73$, $p = 0.07$). There was no correlation between original and foster yolk androgen levels ($r = -0.31$, $N = 19$, $p = 0.19$). Original yolk androgen levels of eggs from 26 nests could be analysed, but due to problems in cross-fostering, both original and foster androgen levels (as estimated from the third egg) could be obtained for 19 nests only. We decided to use the sum of the concentrations of A4 and T (total androgen concentration, pg/mg) in the analyses as a measure of total hormone available for binding to either androgen or oestrogen receptors. This method was chosen because A4 is the total source (precursor) available for hormone conversion to active androgens [testosterone and dihydrotestosterone, (DHT)] and estradiol (E2), T is also a source for DHT and E2, and DHT has a higher affinity to androgen receptors than T (reviewed by e.g. Norris, 1996, Groothuis and Schwabl, 2008). In addition, A4 and T levels are positively correlated ($r = 0.50$, $N = 26$, $p = 0.01$). However, whether the effects of the two hormones differ is uncertain, and their affinity to receptors is likely to differ (e.g. Groothuis and Schwabl, 2008). Therefore, we also

examined the effects of each hormone separately and report the results when they clearly differ for the two hormones.

Yolk androgen manipulation experiment

This experiment was conducted on a different set of nests (N = 66) than the cross-fostering experiment. Nest boxes were checked every other day to determine laying dates. On the estimated day of clutch completion (i.e. day of laying of the sixth egg), each clutch was randomly assigned to either the control or the androgen-manipulation group. All the eggs of a clutch were treated in the same way. The eggs were replaced with dummy eggs for the time of the injections. In the androgen-manipulation group (N = 36 nests), the eggs were injected with 14.4 ng of testosterone (Fluka) and 50.8 ng of androstenedione (Fluka) dissolved in 4 µl sesame oil. In the control group (N = 30 nests), eggs were injected only with 4 µl of sesame oil. The amount of injected androgens was calculated using previous data on natural yolk androgen levels from the same population (T mean: 14.2 ng/yolk, SE 0.4 ng/yolk, maximum: 28.8 ng/yolk; A4 mean: 60.3 ng/yolk, SE 1.5 ng/yolk, maximum 111,1 ng/yolk; B. Doligez and B. Tschirren, unpubl. data). The amount injected corresponds to the difference between mean and maximum values of androgens per yolk, which ensured that final yolk androgen level in the androgen-manipulated clutches was on average at the upper limit of the natural range. The original androgen concentration in the manipulated clutches was not determined, as this would have required removing an egg and thus changing brood size, which could have had substantial effect on the parental feeding rates. The position of the yolk was visualized using a light source positioned beneath the egg. The surface of the egg was first cleaned with 95% ethanol and a small hole was made using a disposable 27 G needle. The oil-vehicle was injected into the yolk using a 25 µl Hamilton syringe (702RN) and 26 G needle. After the injection, the hole in the egg shell was sealed with a drop of tissue adhesive (Vet-Seal, B. Braun Medical, Switzerland) or with a patch of a flexible wound film (Opsite, Smith & Nephew, UK). The eggs

were returned to the nest immediately after the injections. Nests were checked on the following day and the seventh egg was injected if present, according to the clutch treatment. This method for manipulating yolk androgen levels has been successfully used and validated in previous studies (e.g. Tschirren et al., 2005). The hatching success of injected eggs did not differ between the control group (79.74 %) and the androgen-manipulated group (79.77 %, Wilcoxon test: $p = 0.94$, $N = 66$). The natural hatching success of non-injected clutches (excluding deserted and predated nests, where no egg hatched) in this population is 92.0 % (unmanipulated nests of years 1997-2002, N = 1677, B. Doligez and L. Gustafsson, unpubl. data)

On day 9 after hatching, parental feeding rates were recorded with digital video cameras for two hours (SONY Handycam DCR-SR52). If parents were giving frequent alarm calls at the beginning of the recording, feeding rates were estimated from the time when parents stopped alarming and made the first visit back to the nest. Video recordings were watched blindly, i.e. the observers were unaware of nest treatment. As for the cross-fostering and playback experiments, parents were caught, weighed and their age was determined. We tested whether feeding data from the two experiments obtained at different nestling ages (6 and 9 days for the cross-fostering and injection experiment, respectively) are comparable by analysing both 6 days and 9 days post hatching feeding rates in a sample of nests. There was a significant, positive correlation in the male share of feedings between day 6 and day 9 ($r_s = 0.67$, $p = 0.0003$, $N = 25$), and the average male share of feedings was equal at these two measuring points (average male share of feedings: day 6 = 49.4%; day 9 = 49.0%), which strongly indicates that the division of the labour within a pair is fairly constant over this 3-day period.

All experiments were conducted under licences from the Swedish National Board for Laboratory Animals and the Bird Ringing Centre of the Swedish Museum of Natural History (Stockholm, Sweden). The experimental protocols adhered to the standards of US National Institute of Health.

236

237 *Statistical analysis*

238 All statistical analyses were performed with SAS 9.0 reference. Nests where only one
239 parent was observed feeding, or where the age of one parent was unknown (some males could not
240 be caught) or uncertain, were not used in the analyses, which reduces the sample size.

241 In the cross-fostering experiment, we analysed the male's proportion of the total
242 number of feeding visits in relation to yolk androgen concentration (as estimated from the third egg)
243 in original and foster eggs, using a general linear model. We also included female and male age as
244 explanatory variables to control for possible age effects. Laying date, brood size and time of the day
245 when the observations were started were added as covariates in this as well as in all subsequent
246 models. In a second step, we included egg mass and yolk mass in the model to test whether they
247 were associated with male share of feeding activity. The interaction between female and male age
248 could not be tested due to small sample size in the yearling female - yearling male category (sample
249 sizes: yearling females = 3, older females = 13, yearling males = 2, older males = 14).

250 In the androgen-manipulation experiment, we analysed parental feeding rates in
251 relation to androgen-manipulation treatment using a general linear mixed model. We tested the
252 influence of treatment (control or androgen-manipulated), sex, age of the parent and their
253 interactions on the number of feeding visits per hour (i.e. feeding rate). Sample sizes were: yearling
254 females = 12, older females = 32, yearling males = 14, older males = 30. Nest identity (nested
255 within treatment) and forest patch were included as random effects to control for the non-
256 independence of parental responses within a pair and for patch effects.

257 We used a backward model selection procedure, removing interactions, covariates and
258 main effects starting with the least significant. To confirm the non-significance of the removed
259 interaction terms and main effects, each term was added to the final model separately. Satterthwaite

approximation was used to determine denominator degrees of freedom (Littell et al., 1996) and normality of the residuals was checked.

Results

Male feeding rate in the cross-fostering experiment

Average female feeding rate (± SD) was 12.0 ± 5.2 feeding visits / hour (N = 31) and average male feeding rate was 11.4 ± 4.5 (N = 28). The proportion of feeding visits by the male increased with increasing yolk androgen levels in the foster eggs (as measured from the third egg; $F_{1,12} = 8.98$, $p = 0.011$, Fig. 1, average proportion of male feedings (± SD): 49.4 ± 17.9%), controlling for laying date ($F_{1,12} = 10.17$, $p = 0.008$, ^{2} ± SE = -0.037 ± 0.011), and time of the day ($F_{1,12} = 4.38$, $p = 0.058$; ^{2} ± SE = 0.031 ± 0.015). The proportion of feeding visits by the male was not related to the original yolk androgen levels ($F_{1,11} = 0.24$, $p = 0.63$). Separate analyses of both androgens revealed that male proportion of feeding visits was related to yolk A4 ($F_{1,12} = 12.33$, $p = 0.004$) but not to yolk testosterone levels ($F_{1,12} = 0.07$, $p = 0.79$). In order to find out whether the increase in the proportion of male feeding visits was explained by an increase in male feeding rate or a decrease in female feeding rate, we analysed the absolute feeding rates of both sexes. Male feeding rate increased with increasing foster yolk androgen concentration ($r = 0.45$, $N = 20$, $p = 0.042$), whereas no significant correlation was observed in females ($r = -0.24$, $N = 20$, $p = 0.30$). There was an interaction between parental sex and foster yolk androgens ($F_{1,28} = 4.26$, $p = 0.048$; males: ^{2} ± SE = 0.033 ± 0.028; females: ^{2} ± SE = -0.028 ± 0.050). These results are in accordance with the hypothesis that females manipulate males through a yolk androgen-dependent nestling signal, to which only males react, and / or a maternal effect that correlates with yolk androgen concentrations in the eggs. Age of the parents did not affect the proportion of feeding visits by the male, and there were no significant interactions between female or male age and original or foster yolk androgen

concentration (all p-values >0.08). Finally, the proportion of feeding visits by the male was not related to egg mass ($F_{1,11}$ = 0.41, p = 0.53) or yolk mass ($F_{1,11}$ = 0.32, p = 0.58). Thus the relationship between yolk androgen content in foster eggs and male feeding effort can not be explained by egg or yolk mass.

Feeding rates in the yolk androgen-manipulation experiment

There was no effect of the androgen-manipulation on parental feeding rates and no interaction between treatment and sex (interaction treatment x sex: $F_{1,42}$= 0.46, p = 0.50; treatment: $F_{1,41}$ = 0.38, p = 0.82; sex: $F_{1,42}$ = 1.56, p = 0.21, Fig. 2.). Feeding rates of yearling and older individuals did not differ between androgen-manipulated and control group (interaction age x treatment: $F_{1,82.6}$ = 0.12, p = 0.73; age: $F_{1,82.3}$ = 0.02, p = 0.89). Feeding rate increased with brood size (controlled for in the above analyses; $F_{1,37}$ = 13.87, p < 0.001, ² ± SE = 2.336 ± 0.627). All other interactions and covariates were non-significant (all p-values > 0.12).

Discussion

Do females manipulate males through differntial yolk androgen deposition?

The results of the cross-fostering experiment suggest that, in the collared flycatcher, sexual conflict over parental care may be influenced by the female via differential allocation of yolk androgens or other egg components (e.g. carotenoids, lipids) that are correlated with the yolk androgen content of the egg. The correlation between the proportion of feeding visits by the male and yolk androgens in the foster eggs was explained by an increase in male absolute feeding rate, which suggests that offspring traits associated with high yolk androgen levels make specifically males to increase their effort . However, when yolk androgen levels were experimentally elevated, males feeding nestlings

originating from androgen-manipulated eggs did not feed at a higher rate than those feeding control clutches. This indicates that the androgen levels *per se* did not affect male effort.

It could be argued that the effects of androgens on the chicks may depend on synergistic interactions with other egg components (e.g. Groothuis and von Engelhardt, 2005), or that exogenous A4 and T in the yolk could be differentially metabolized by steroidogenic enzymes than endogenous steroids. In this case, experimental elevation of only the androgen levels using exogenous hormones might not have had an effect on the chicks. This is however unlikely, because experimental elevation of yolk androgen levels following the same protocol as ours had a sex-specific effect on chick growth in another set of nests in our study population (Pitala et al., *in press*). This shows that exogenous androgens do have an effect on the chicks. Thus the result of the androgen-manipulation allows us to conclude that yolk androgens do not appear to mediate sexual conflict over parental care in the collared flycatcher (potential explanations for the association between yolk androgens and male investment in the cross-fostering experiment are discussed below).

To our knowledge, only one study thus far has examined whether yolk androgens may play a role in mediating sexual conflict over parental investment. In great tits, only females adjusted their parental effort to androgen-mediated nestling traits, indicating that male manipulation by the female via yolk androgens is also unlikely in this species (Tschirren and Richner, 2008). Thus no empirical evidence currently supports the hypothesis that females could successfully manipulate male investment through yolk androgens. Selection may have favoured males' reduced susceptibility to yolk androgen-mediated nestling traits, allowing them to resist the manipulation by females (Tschirren and Richner, 2008). In an evolutionary conflict of interests, such as sexual conflict over parental investment, an "arms-race" is expected, with each parent trying to minimise costs and maximise benefits (e.g. Arnquist and Rowe, 2005). Thus males may be expected to evolve responses to specific offspring signals that cannot be manipulated by females and/or ignore signals

that can be manipulated by females (Müller et al., 2007a). Indeed, males and females are known to use different cues, for example nestling sex, size, position, vocal or visual components of begging, in adjusting their feeding effort (e.g. reviewed in Lessells 2002, Müller et al., 2007a). The evolution of male counter-responses to female manipulation will depend on the balance between benefits (avoiding manipulation by the female) and costs (e.g. reduced reproductive success) of ignoring the manipulated nestling traits. Unfortunately, empirical testing of the hypothesis that past manipulation by females has selected males to ignore certain offspring signals may prove very difficult. However, Lessells (2006) pointed out that, in general, manipulative behaviour in sexual conflict over parental investment may be rare, as the benefits may be small and the costs could be substantial. Thus, sexual conflict over parental investment may not generate rapid evolutionary change and antagonistic coevolution.

Relationship between male investment and yolk androgens in the cross-fostering experiment

The results of the androgen manipulation experiment suggest that yolk androgens *per se* do not mediate sexual conflict over parental investment. Therefore, the correlation between male share of parental care and yolk androgen level of fostered clutches in the cross-fostering experiment (Fig. 1) probably arises through a different pathway. For instance, the amount of yolk androgens in the egg could be correlated with another egg component that females could use to manipulate male effort. The amount of yolk androgens has indeed been found to correlate with, for example, the amounts of antioxidants and immunoglobulins in the yolk (e.g. Royle et al., 2001; Groothuis et al., 2006; Török et al., 2007). The role of egg components other than androgens in mediating sexual conflict over parental investment however remains to be investigated. The amount of yolk androgens could also be positively correlated with the genetic quality or condition of the parents and subsequently with the quality of the offspring, to which the parents respond. It is yet possible that the quality of the offspring depends on the quality of their natal territory. A correlation with yolk androgen levels

could then arise for example if higher quality territories are more fiercely defended, leading to higher androgen levels in female circulation and subsequently to higher yolk androgen levels in the eggs (e.g. Schwabl, 1997). Thus the positive correlation between male parental care and yolk androgens could arise from males responding to the overall quality of the offspring.

Conclusions

The results of our experiments indicate that female collared flycatchers in our population do not differentially allocate yolk androgens to manipulate males into higher parental investment. However, they might be able to manipulate males via other maternal effects correlated with yolk androgens. Identifying the egg components or chicks characteristics that appear to differentially influence the effort of male and female parents would be an important next step towards a better understanding of the potential of maternal effects in mediating sexual conflict over parental investment.

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601 Fig. 1. Male's proportion of feeding visits in relation to total androgen concentration in eggs (as
602 estimated from the third egg of the clutch) of fostered nestlings (N = 16, $F_{1,12}$ = 8.98, p = 0.011).
603 Residuals are from a model in which we controlled for the effects of laying date and time .
604
605 Fig. 2. Male and female feeding rates (per hour; mean, 1SD) in control and androgen manipulated
606 nests (interaction treatment x sex: $F_{1,42}$= 0.46, p = 0.50). Sample sizes are indicated above the bars.